Pilot Study on the Effects of Seismic Air Gun Noise on Lobster (*Homarus americanus)*

J.F. Payne, C.A. Andrews, L.L. Fancey, A.L. Cook and J.R. Christian

Science Branch
Fisheries and Oceans Canada
P.O. Box 5667
St. John's NL Canada A1C 5X1

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PILOT STUDY ON THE EFFECTS OF SEISMIC AIR GUN NOISE ON LOBSTER (*HOMARUS AMERICANUS*)

by

J.F. Payne[1], C.A. Andrews[1], L.L. Fancey[1], A.L. Cook[2] and J.R. Christian[3]

[1]Science Branch
Fisheries and Oceans Canada
P. O. Box 5667
St. John's NL A1C 5X1

[2]Oceans Ltd.
85 Lemarchant Road
St. John's NL A1C 2H1

[3]LGL Ltd.
388 Kenmount Road
St. John's NL A1B 4A5

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ABSTRACT

Payne, J.F., Andrews, C.A., Fancey, L.L., Cook, A.L., and Christian, J.R. 2007. Pilot study on the effect of seismic air gun noise on lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci. 2712: v + 46.

The question of impacts of seismic surveys on fish mortality and morbidity as well as potential for effects on fish behavior continues to be of concern for the fishing sector as well as industrial and management interests in relation to oil and gas development in the offshore. Effects on fishing success have been noted in a few studies indicating potential for temporary displacement of at least some fish populations. However (and as also indicated by other researchers) displacement through scaring of fish for a number of hours or a day should not, in perspective, pose a major problem. Adults, juveniles and eggs may also suffer immediate mortality within a few meters of a sound source. However it is sub lethal effects which are most difficult to deal with and serious physiological and anatomical damage may also be occurring at much greater depths in the field leading to a variety of injurious effects. There is virtually no information in this area and it needs to be addressed if only for assurance.

Lobster is one of the most commercially important species in Atlantic Canada and it is important to have some knowledge on whether seismic poses a threat to lobster populations, and now more so, given recent interests for expanding seismic surveys in such areas as the lobster rich and more shallow waters of the Gulf of St. Lawrence. Some pilot studies have now been carried out in the laboratory and field investigating the potential for effects of seismic on lobster health. The basic thrust of the studies was to explore for changes in various biological endpoints and identify those (if any) which might then require further assessment in a more comprehensive manner. A number of endpoints were assessed in animals exposed to a 'low level" exposure ~202 dB peak-to-peak and a "high level" exposure ~227 dB peak-to-peak. The endpoints included (a) lobster survival (b) food consumption (c) turnover rate (d) serum protein (e) serum enzymes and (f) serum calcium. A small histopathological study was also carried out on lobsters from 1 of the 5 trials. Observations were often made over a period of a few days to several months.

Exposure of lobster to very high as well as low sound levels had no effects on delayed mortality or damage to mechanosensory systems associated with animal equilibrium and posture, as assessed by turnover rates. There was also no evidence for loss of legs or other appendages. However sub-lethal effects were observed with respect to feeding and serum biochemistry with effects sometimes being observed weeks to months after exposure. A histochemical change was also noted in the hepatopancreas of animals exposed 4 months previously, which may be linked to organ 'stress'. These initial studies were meant to be exploratory in nature and caution is warranted about over interpretation. However, they do point to the need for more comprehensive

studies regarding the potential for seismic surveys to affect lobster. Studies on moulting and effects on egg development and animal behavior are recommended. Such studies can be carried out in a cost effective manner through laboratory and small scale field experiments as used in the pilot investigations reported here.

The difficulties in most instances of resolving seismic mediated impacts through studies on fish populations, short of major or catastrophic impacts, is also briefly discussed.

RÉSUMÉ

Payne, J.F., Andrews, C.A., Fancey, L.L., Cook, A.L., and Christian, J.R. 2007. Pilot study on the effect of seismic air gun noise on lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci. 2712: v + 46.

La question des incidences des levés sismiques sur la mortalité et la morbidité chez le poisson ainsi que leurs effets possibles sur le comportement des poissons reste préoccupante pour le secteur des pêches ainsi que pour les industriels et les gestionnaires dans le secteur de la mise en valeur des ressources pétrolières et gazières extracôtières. Des effets sur le succès de pêche ont été signalés dans quelques études qui indiquent une possibilité de déplacement temporaire, à tout le moins de certaines populations de poissons. Cependant (comme l'indiquent également d'autres chercheurs) des déplacements de poissons effrayés pendant un certain nombre d'heures ou une journée, ne devraient pas poser un problème majeur lorsque mis en perspective. Les adultes, les juvéniles et les œufs peuvent également mourir immédiatement dans un rayon de quelques mètres d'une source sonore. Ce sont toutefois les effets non mortels qui sont les plus difficiles à cerner et de sérieux dégâts physiologiques et anatomiques peuvent également être causés sur le terrain à de beaucoup plus grandes profondeurs ce qui peut entraîner toute une gamme d'effets nuisibles. Il n'existe à toutes fins utiles aucune information à ce sujet qui doit être abordé, ne serait-ce qu'à des fins de vérification.

Le homard est l'une des espèces commerciales les plus importantes au Canada atlantique et il est primordial de savoir si la prospection sismique menace les populations de homard et d'autant plus pressant de le déterminer compte tenu de l'intérêt récent manifesté pour l'extension des levés sismiques aux régions aux eaux moins profondes et riches en homards du golfe du Saint-Laurent. Certaines études pilotes des effets potentiels des levés sismiques sur la santé du homard ont été effectuées en laboratoire et sur le terrain. Leur objectif fondamental était la recherche de changements de divers effets biologiques ultimes et, le cas échéant, l'identification de ceux qui pourraient mériter une évaluation plus poussée et plus globale. Un certain nombre d'effets ont été évalués chez des animaux exposés à de «faibles intensités» sonores d'environ

202 dB de crête-à-crête et à de fortes intensités d'environ 227 dB de crête-à-crête. Les effets ultimes évalués ont été ceux sur a) la survie du homard, b) la consommation de nourriture, c) le taux de renouvellement, d) les protéines sériques, e) les enzymes sériques et f) le calcium sérique. Une brève étude histopathologique a en outre été menée sur des homards utilisés pour l'un des cinq essais. Les observations portaient souvent sur des intervalles de quelques jours à plusieurs mois.

L'exposition des homards à de très fortes ainsi qu'à de faibles intensités sonores n'avait aucun effet sur la mortalité différée et ne causait aucun dommage aux systèmes mécanosensoriels associés à l'équilibre et à la position des animaux d'après des évaluations fondées sur le taux de renouvellement. Aucune indication de perte de pattes ou d'autres appendices n'a été relevée. Cependant, des effets non mortels ont été observés en ce qui a trait à l'alimentation et à la biochimie sérique, parfois de quelques semaines à quelques mois après l'exposition. Un changement histochimique a en outre été observé dans l'hépatopancréas d'animaux exposés 4 mois plus tôt, et il pourrait être relié au «stress» ou à l'alimentation. Ces études initiales se veulent exploratoires et la prudence est de mise à l'interprétation des résultats. Cependant, elles soulignent la nécessité d'études plus exhaustives des possibilités d'incidences des levés sismiques chez le homard. Des études de l'exuviation et des effets sur le développement des œufs ainsi que sur le comportement animal sont recommandées. Ces études pourraient être menées de manière rentable par l'exécution d'expériences à petite échelle en laboratoire et sur le terrain du genre de celles utilisées dans le cadre des études pilotes ici signalées.

Les difficultés que pose, dans la plupart des cas, la détermination des incidences médiatisées des levés sismiques, autres que les incidences majeures ou catastrophiques, par l'exécution d'études des populations de poissons sont aussi brièvement abordées.

INTRODUCTION

Over the past few years, considerable interest and controversy has arisen over the potential effects of anthropogenic sounds in the marine environment and seismic surveys carried out during exploration for oil and gas deposits have come in for special attention. Seismic surveys involve the deployment of intense sound producing air guns from a survey vessel either singly or more commonly in multiple arrays with guns being shot repeatedly, such that an array can produce thousands of shots over a 24 h period.

Until recently most concerns about seismic surveys revolved around concerns about impacts on the behavior and physiology of marine mammals (e.g. National Research Council 2005). However sounds that affect marine mammals also have the potential to affect fish and invertebrates. Effects on fishing success have been noted in a few studies indicating potential for temporary displacement of some fish populations (e.g. Engås et al. 1996; Hirst and Rodhouse 2000). Experimental evidence has also been produced demonstrating that juveniles and eggs of fish may suffer immediate mortality within a few meters of seismic guns (reviewed in Payne 2004; also e.g. Saetre and Ona 1996). However in addition to immediate mortality, serious physiological and anatomical damage may also be occurring in adult animals in the field leading to effects such as delayed mortality, greater susceptibility to predation, lowered resistance to disease or impaired egg quality. A few examples of this have been reported. For instance, in an opportunistic study with snow crab, egg development was shown to be delayed in extruded eggs that had been exposed at close range 3 months previously (Christian et al. 2003) indicating the value of knowing the approximate sound/energy level which would not produce adverse effects. Pearson et al. (1994) noted that seismic exposures had little or no effect on the survival and development of crab larvae. This was an important observation but earlier stages of egg development have been shown in model systems to be more sensitive to "stress". McCauley et al. (2003) also noted a potential for delayed anatomical damage to the ears of fish in an experiment mimicking relatively high exposures from a seismic vessel.

Overall, the issue of the potential for seismic sounds to produce adverse sub-lethal effects in fish and other aquatic organisms presents a problem for regulatory, industrial and scientific interests since there is essentially no data to address the issue in any meaningful manner, as also recognized by others (e.g. Popper 2003). The major data gaps surrounding seismic effects have also been noted in a DFO habitat status report (DFO 2003).

Confounding perceptions about the effects of seismic on fish is the often made comment that there are no effects at the population level. It is a well known precept in fisheries science that short of major effects, it would be very difficult to measure the impact of elements such as seismic, chemical contamination etc. on fish populations due to natural variability and/or such

factors as fishing mortality (Payne 2007). This point has been specifically demonstrated in relation to seismic in an Australian study (Parry and Gason 2006) which found no effects on lobster catches in regions where seismic surveys had been carried out. However, it is important to point out that a seismic induced mortality rate in the 50% range would have been required before a seismic impact could have been resolved from other factors.

Likewise, should field studies with snow crab or lobster be carried out to attempt to resolve any seismic induced effects on populations after a seismic survey, impacts would likely have to be quite large, to differentiate any seismic induced effects due to factors such as varying mortality, animal movement and fishing practices. Considering movement alone, both lobster and snow crab have been recorded to travel tens of kilometers (and often more) in a year (Lawton and Lavalli 1995; Bailey and Jamieson 1990). The problem would also be greatly compounded if effects occurred for instance, in small moulting animals which would not be expected to recruit into a fishable population for a number of years.

Accordingly, there is presently a need to explore distance/exposure relationships for potential physiological and pathological effects with representative fish and invertebrate species. Since seismic surveys are carried out in the open ocean it can be asked how questions about defining physiological and pathological effects can be addressed in a practical and economical manner in relation to exposure levels, short of individual field experiments being carried out in conjunction with surveys, and which would cost millions. An approach to this is through experiments in large tanks or via small scale field trials in which various exposure levels can be investigated through use of single (or a few) guns having appropriate source levels (Thompson et al. 2000).

Lobster is one of the most commercially important species in Atlantic Canada and it is important to have some knowledge on whether seismic surveys may pose a risk to lobster populations. Noted in this regard is the mounting interest with respect to seismic surveys in the relatively shallow waters of the Gulf of St. Lawrence which has extensive and rich lobster grounds. Some pilot studies have now been carried out investigating the potential effects of seismic surveys on lobster health. The basic thrust of the studies was to explore for changes in various biological endpoints and identify those (if any) which might then warrant further assessment in a more comprehensive manner.

Biological indices including animal survival, feeding and turnover rates were investigated. A few hematological indices which can be important indicators of physiological stress were also assessed in some trials and at varying periods after the sound exposures. This included serum protein, serum aminotransferase (AST) and creatine kinase (CK), and serum calcium. Generally, change in total protein can indicate abnormal release of protein from body compartments, abnormal clearance from blood (hemolymph) or effects on

protein synthesis. Serum enzymes are widely used in human and veterinary medicine (Ettlinger and Feldman 1995) to diagnose damage to organs and tissues. Enzymes can leak into blood in elevated concentrations following damage. (e.g. Suber 1994; Kaneko et al. 1997) and although commonly used in human and veterinary medicine, serum enzymes are also increasingly being used in studies with fish and shellfish. (Noga 2000; Mathieu et al. 2001; Christian et al. 2003). Various serum parameters including serum enzymes have also recently been studied in whales in relation to sound exposures (Romano et al. 2004). Alternatively, instead of enzyme elevation due to tissue damage, as commonly found in vertebrates, enzyme levels may decrease in the blood of shellfish indicating hemodilution and signaling osmoregulatory or other physiological disturbance.

Histopathological studies were also carried out on the ovary and hepatopancreas of lobsters from one of the trials. Histopathology is a cornerstone of human and veterinary medicine and is increasingly being used to assess tissue and organ abnormalities in aquatic organisms (e.g. Hinton et al. 1992; Myers and Fournie 2002; Payne et al. 2003).

Catecholamines, including norepinephrine, epinephrine and dopamine which act as hormones and neurotransmitters and are released into the serum as an initial response to stress, were also investigated (e.g. Romano et al. 2004). There is considerable interest in these compounds as indicators of stress in invertebrates as well as vertebrates, but the values were too low in lobster for reliable analysis, including in seismic exposed animals, and studies on catecholamines were terminated after initial trials.

MATERIALS AND METHODS

ANIMAL SOURCE AND MAINTENANCE

Animals were obtained from commercial outlets and stock supplies were fed shucked mussels and capelin. Animals were held in 2000L aquaria which were supplied with aerated, flow-through, ambient temperature seawater.

EXPOSURES

Exposures were carried out with a 10 in^3 sleeve gun in the laboratory and a 40 in^3 sleeve gun in the field. Guns were from Texas Instruments. The low level exposures were carried out in a large aquarium, (inside dimensions: 3.63 m length X 2.39 m width X 1.27 m depth with water depth 1.13 m), in the laboratory while the high-level exposure was carried out in the field.

Exposures in the laboratory involved suspending the gun about midway in the aquarium and placing the caged animals at the end. In the field, the gun was lowered to a depth of 2 m from a 50 ft fishing vessel with the animals being positioned directly below the gun at 4 m depth. Received levels were measured in the laboratory while levels in the field were interpolated (assuming spheric spreading) through back calculation of levels recorded approximately 20 m distant. Control animals in the laboratory and field studies were handled in the same manner as the experimentals except for the sound exposures. Conditions were satisfactory for obtaining the desired sound levels of ~200 dB p-p for the low level exposures and ~230 dB p-p for the high level exposures. Upon exposure, lobsters were maintained in aquaria at DFO for long term observation and sampling.

Hydrophone specifications were as follows: Manufacturer: Reson; Model: TC 4014; Usable Frequency Range: 15Hz – 480 kHz; Horizontal Directivity Pattern: Omni directional; Vertical Directivity Pattern: 270 deg +/-2 dB at 100 kHz Receiving Sensitivity: -186 dB +/-3 dB Operating Depth: 900 m.

LOBSTER HEALTH

A number of endpoints were assessed including (a) lobster survival (b) food consumption (c) turnover rate (d) serum protein (e) serum enzymes and (f) serum calcium. A small histopathological study was also carried out on lobsters from 1 of the 5 trials. Observations were made over a period of a few to several months, depending on the specific trial. Systematic periodic analysis of endpoints was outside the scope of the pilot study – per additional costs. In the food consumption studies, lobsters were fed a weighed amount of shucked mussels and the amount of food remaining after various periods of time, again depending on the trial, was collected and weighed. Turnover rate was assessed by measuring the time required for animals to maintain a normal upright position upon placement on their "back". For studies on serum parameters, ~2 mL hemolymph was collected from the abdomen close to the carapace and placed on ice. The hemolymph was then centrifuged at 2000 X g for 3 minutes to precipitate any remaining hemocytes and the serum was stored at -60°C.

SERUM ENZYMES

Aspartate aminotransferase (AST) and creatine kinase (CK) were determined using colorimetric endpoint methods employing coupled reactions. Kits were supplied by Sterling Diagnostics, Inc. Calcium was also determined colorimetrically through its reaction with arsenazo III with kits supplied by JAS Diagnostics, Inc. Protein was determined according to Lowry et al. (1951).

HISTOPATHOLOGY

Samples of the hepatopancreas and ovary from Trial 2 were fixed in Gendre's Fluid for histopathological studies. Samples were processed using an Autotechnicon Tissue Processor according to the general procedures of Lynch et al. (1969). A graded ethanol series was used to dehydrate the samples with clearing in three changes of Citrisolv™. Tissues were then impregnated with three changes of molten embedding media , Tissue Prep™. Sections were cut at 6µ and stained with (a) Mayer's Hematoxylin and Eosin (b) Periodic Acid Schiff and (c) Papanicolaou.

Biological stains were from Sigma Chemical Compa y and the staining of tissues followed the general procedure supplied with the kits. The theory and practices associated with the use of the various stains can be found in Luna (1968) or Bancroft and Gamble (2002).

Slides were examined under different magnifications by light microscopy for any microstructural differences between the control and exposed group.

STATISTICS

The unpaired T test or the Mann-Whitney Rank Sum test was used to compare mean differences between the control and exposed groups.

RESULTS

MEASUREMENTS

Measurements were carried out on three different occasions in the laboratory to monitor the received levels at the cage site in the aquarium where the animals were exposed (Table 1). Peak to peak pressures averaged ~202 dB with energy densities (dB re 1µ Pa^2/Hz) ranging from 144 to 169. Three separate measurements were also carried out in the field. Due to sound saturation at the cage site, animal exposures were back calculated from levels received at a distance of approximately 23 meters. The source levels (dB re 1µPa) averaged 230 dB peak-to-peak (229, 230, 230) with peak energy densities of 175 @ 26 Hz, 187.4 @ 26 Hz and 186.2 @ 25 Hz. Since the animals were placed 2 m from the gun, the back calculation provided an average received level of approximately 227 peak-to-peak and an average peak energy density of 187. The average density calculation assumes subtracting ~40 dB from peak-to- peak levels (Scientific Committee on Antarctic Research 2002).

ANIMAL SURVIVAL

Survival of exposed animals was somewhat higher in Trial 1 (Fig. 1) and lower in Trials 2 and 3 (Fig. 2 and 3) with essentially no change between the two groups in Trials 4 and 5. (Fig. 4 and 5) A somewhat high level of mortality was observed in both groups in Trial 1 (Fig. 1) but this Trial was carried through the summer and autumn and relatively higher rates of mortality are not uncommon on holding animals under warm water conditions.

Given the variability in survival with the trials indicating either slightly positive, negative or no effects of exposure, it was of interest to sum the survival rate in all the trials (Fig. 6) This summation gave equivalent survival rates for the 235 lobsters (118 control and 117 experimental) used in the 5 trials.

FOOD CONSUMPTION

Food consumption measured in lobsters in Trial 1, 15 days post-exposure (the only period in which food consumption was measured in this initial trial) (Fig. 7) was observed to be increased in the exposed animals. Food consumption was likewise found to be increased in exposed lobsters in Trial 2, 69 days post-exposure (Fig. 8a and b). Similar results were obtained in Trials 4 and 5. In Trial 4, food consumption was greater in exposed animals 12 days post-exposure (Fig. 11a and b) and was still noticeably greater 40 days post-exposure, (Fig. 12a and b) with equivalent amounts of food being eaten by control and exposed animals on day 162 (Fig. 13a and b). Likewise in Trial 5, food consumption was observed to be increased in exposed lobsters on both occasions in which feeding was measured 20 days post-exposure (Fig. 14a and b) and 77 days post-exposure (Fig. 15a and b). However, food consumption was found to be decreased in exposed lobsters in Trial 3. A substantial decrease was noted 8 days post-exposure and was still apparent 43 days post-exposure (Fig. 9b and 10b).

TURNOVER RATE

Turnover rates were investigated in Trials 1 (Fig. 16), 2 (Fig. 17) and 4 (Fig 18) on days 142, 65 and 9 days post–exposure respectively. No significant differences were noted between control and experimental groups.

SERUM PROTEIN

Serum protein was measured in Trials 2 (Fig. 19a, b and c), 3 (Fig. 20), 4 (Fig. 21), and 5 (Fig. 22) at various times. In Trial 2 (Fig. 19a) there was indication (P=0.1) of a reduction in protein in exposed lobsters 5 days post

exposure. Statistical significance was reached (P=0.04) 12 days post-exposure (Fig. 19b) with a trend towards reduction still being maintained (P=0.25) 33 days post exposure (Fig. 19c). A reduction in serum protein was similarly noted in Trials 3 (Fig. 20) and 4 (Fig. 21) with a P value of 0.07 being obtained in both trials. No differences in serum protein were obtained between control and experimental animals in Trial 5 (Fig. 22).

SERUM ASPARTATE AMINOTRANSFERASE AND CREATINE KINASE

Serum aspartate aminotransferase (AST) and creatine kinase (CK) were measured in Trials 2 (Fig. 23), 3 (Fig. 24), 4 (Fig. 25) and 5 (Fig. 26). A significant reduction in serum AST (P=0.02) was noted in exposed animals in Trial 2, 5 days post-exposure (Fig. 23a). A trend towards reduced levels was maintained 12 (Fig. 23b) and 33 (Fig. 23c) days post-exposure with a P value of 0.10 being obtained in each instance. AST was not reduced in animals exposed in Trial 3 (Fig. 24) or 5 (Fig. 26) but was reduced somewhat (P=0.12) in animals exposed in Trial 4 (Fig. 25). Somewhat similar results were obtained for creatine kinase. A significant reduction in serum creatine kinase was noted in exposed animals in Trial 2, 5 days post-exposure (P=0.05) (Fig. 27a) with trends towards reduction still being maintained 12 (Fig. 27b) and 33 (Fig. 27c) days post-exposure where P values of 0.11 and 0.18 respectively were obtained. The enzyme appeared not to decrease in the serum of animals exposed to Trial 3 (Fig. 28), but there was indication of a decrease in Trial 4 (Fig. 29) and to a greater extent in Trial 5 (Fig. 30) where P values of 0.29 and 0.17 respectively were obtained.

CALCIUM

A few measurements on serum calcium were also carried out, namely in Trials 2 (Fig. 31) and 4 (Fig. 32). There was indication of a reduction in serum calcium (P=0.20) in animals from Trial 2 examined 5 days post exposure. (Fig. 31a) This reduction was fully apparent 12 days post-exposure (P=0.006) (Fig. 31b). There was also indication of a reduction in serum calcium in animals exposed in Trial 4, 5 days post–exposure (P=0.13) (Fig. 32).

HISTOPATHOLOGY

A limited histopathological study namely on the hepatopancreas and ovary was carried out on lobster from one of the trials. There was no evidence for cellular rupture or necrosis in the hepatopancreas of lobster examined approximately 4 months post exposure nor was there evidence for necrosis or inflammation of the ovary. However, deposits of PAS staining material were

found to a much greater extent in the hepatopancreas of the animals which had been exposed 4 months previously (Fig. 33).

DISCUSSION

ANIMAL SURVIVAL

Unlike dynamite charges which were historically used in seismic survey work and often caused immediate mortality of adult fish and shellfish some distance away, the use of air-guns has circumvented this major environmental problem. However, there remains the more difficult question of delayed mortality, which can not be assessed in the field, except possibly after rather catastrophic mortality events (as noted in the Introduction). The present study did not provide evidence for delayed mortality in lobsters several months after exposure, with some observations extending to 9 months. This was true for the high energy as well as the low energy exposures. There was also no evidence for the production of major external abnormalities such as the loss of legs or other appendages.

TURNOVER RATE

Damage to sensory systems is one of the outstanding concerns surrounding seismic surveys with considerable attention being given to marine mammals. However, concerns have also been expressed in relation to fish and other organisms including crab and lobster. McCauley et al. (2003) provided evidence for anatomical damage to the ears of fish through destruction of hair cells involved in hearing. However, hearing was shown not to be adversely affected in three species of fish subjected to rather high sounds during a field investigation in the MacKenzie River (Popper et al. 2005). The potential for effects on the statocyst or its associated structures, which is critical for geo-orientation and maintaining balance (and possibly "hearing") in crustaceans (Atema and Voight 1995) have also come in for attention. Assessment of the ability of crustaceans such as lobster and crab to maintain normal posture upon placement on their "back" is an important parameter for assessing any significant injury to the statocyst apparatus. Turnover rates were investigated in three separate trials in this study and no differences were observed between control and exposed animals. This supports the hypothesis that seismic surveys do not pose a risk to important geo-orientation and equilibrium functions in lobster.

FEEDING

Feeding is an important indicator of overall physiological fitness or morbidity. It was of interest to note that although feeding differences were not

major, food consumption increased in exposed animals in four out of the five trials carried out. Further, the differences in consumption were often apparent several weeks post-exposure. This effect is suggestive of an overall disturbance in metabolic rate or perturbation of neural/endocrine functions associated with feeding. Interestingly, increase in food consumption is a known manifestation of brain/neural injury in humans (Henson et al. 1993) putatively through effects on the hypothalamus in the case of vertebrates (e.g. Wolf 2006). It is also worth noting that an increase in food consumption has similarly been observed in codfish exposed to seismic in a pilot study (unpublished). However, food consumption was decreased in exposed animals in Trial 3 which were exposed during "high summer" water temperatures which are generally known to be stressful leading to greater mortalities in animals held in the laboratory or commercial holding pens. The question can be asked whether the fairly marked reduction in feeding noted in this particular case could be a reflection of seismic having an added effect on already "stressed" animals with the reduction in food consumption overriding the tendency towards increased food consumption, as noted in the other four trials. This area needs further investigation in relation to the novel observation on the potential for seismic to perturb (putatively) neural/endocrine functions to an extent to affect a physiological endpoint such as feeding. Furthermore, neural disturbances can be diffuse providing potential for effects other than on feeding. Any neurological disturbances would also bring to mind the question whether such disturbances might apply to other species. It is noted in this regard that we have also noted enhanced feeding in codfish exposed to seismic. There is also at this time the "practical" question on whether feeding might be reduced in animals exposed under more stressful warm water conditions such as during late summer and "indirectly" produce injurious effects on such important physiological processes as moulting. Further, there is understanding that "sustained" neural/endocrine effects could also potentially affect moulting, mating or other important physiological processes in a more "direct" manner.

SERUM PARAMETERS

Serum enzymes can be valuable in detecting major organ damage whereby enzymes leak into the blood (hemolymph) in elevated concentrations upon cellular rupture (e.g. Suber 1994; Kaneko et al. 1997). Enzymes are widely used in human medicine for diagnosing heart attacks, liver damage, muscular defects, etc. It is of interest that two enzymes AST and CK which are enriched in hepatopancreas and muscle tissues respectively were not elevated in seismic exposed animals. This was a significant result indicating the absence of any major cellular rupture or necrosis being effected by seismic including under very high exposure conditions. Similar results were obtained in studies with snow crabs (Christian et al. 2003) including in animals examined several months after exposure (Mathieu 2003).

However, there was evidence of a decrease in serum enzymes in some trials indicating the possibility of hemodilution or uptake of excess water by the animals. A similar decrease in serum protein and calcium was also noted in some trials indicating a potential for disturbance to osmoregulation. Observations on decrease in serum protein could suggest disturbance to protein synthesis, release of protein from various body compartments or its clearance from hemolymph, but a general increase in hemodilution could also cause such an effect. The observation on calcium reduction also suggests a hemodilution or osmoregulatory disturbance. Altogether, the results suggest a potential for osmoregulatory disturbance in lobsters exposed to seismic.

HISTOPATHOLOGY

Histopathology forms the basis for diagnosing various health conditions in humans and is increasingly being used in assessing the health of aquatic animals. Studies were carried out on the hepatopancreas and ovary of female lobsters from one of the trials. No structural differences denoting cell or tissue rupture, necrosis or inflammation, as assessed by light microscopy, were noted in hepatopancreatic tissues of control and exposed animals. Similar results were reported by Christian et al. (2003) in snow crab exposed to seismic including with relatively high exposure levels. Histological sections were read after using three different staining procedures: Hemotoxylin and Eosin, Periodic acid Schiff and Papanicoloau. There was also no evidence for tissue "necrosis" or infiltration of hemocytes (i.e. inflammation-like phenomena) in the ovary. Interestingly however, evidence was provided through the PAS staining procedure for elevated deposits of carbohydrate in the hepatopancreas of exposed animals (Fig. 33). Such deposits were mostly removed through pre-incubating sections with amylase, suggesting that the deposits were glycogen. Further, this effect was noted approximately 4 months post-exposure. Cells known as R cells (resorptive cells) are important in the absorption and storage of glycogen and lipid in decapod crustaceans. (Al-Mohana and Nott 1987, 1989).

Toxic stress has been shown to result in the accumulation of glycogen and/or lipid in model mammalian systems and humans. It has also been reported on occasion in relation to toxic stress in fish. (e.g. Payne et al. 1988). Such abnormal accumulations are believed to be due to disturbance in cellular processes connected with synthesis and secretion. Given general observations regarding abnormal accumulation of lipid and/ glycogen in vertebrates in connection with (chemical) stress, and the observations here, it would be useful to further assess this particular effect in lobster to determine if the effects observed here were due to organ "stress".

SOUND MEASUREMENTS

Sound measurements included peak-to-peak pressures as well as the density spectrum. Peak-to-peak pressures are the standard measure of air gun array power used by the geophysical industry. Peak-to-peak pressures were around 202 dB re 1µ Pa in the low level exposures and 227 in the high level exposures. Assuming spherical spreading, guns having a source level of 240 dB would be expected to produce sound pressures around 200 dB at a depth of 104 m in the water column (Fig. 34) or roughly the depth of water on the Grand Banks for instance. Similarly, a gun having a source level of 250 dB would be expected to produce sound pressure levels around 200 dB down to over 300 m in the water column. Thus an exposure around 200 dB is reasonable in any initial exploratory studies (with lobster or other organisms), whose purpose is to explore for particular physiological and pathological endpoints which might be affected by seismic exposure. These initial studies can then provide a basis for identifying those for which there might be a need/no need to pursue in more detail. Or by extension, if different types of sub-lethal effects are observed, this might pin-point a need for a specific study on a more general physiological endpoint. The potential for effects on moulting and egg development can come to mind in this regard, on the basis of the initial studies with lobster. Caution is warranted in comparing sound metrics but it was of interest to note that the mean squared pressure levels in the study which reported anatomical damage to fish ears (McCauley et al. 2003) peaked around 180 dB but also sometimes reached approximately 188-190 dB. Following the formula dB (p/p) = dB (msp) + 19, peak to peak pressures up to 209 dB (190 dB +19 dB) would have occasionally occurred in the study. By comparison the peak to peak pressures in the study with lobster were around 202 dB.

An exposure level around 227 dB was also used in the study. The question can be asked: why such an exposure level which is not really relevant under field conditions? Very high exposure levels in which negative results are obtained can provide important information. For instance, if a particular effect is not observed at very high exposure levels, it is difficult to make a case for that effect at much lower levels. Noted in this study were the lack of effects on delayed mortality, loss of appendages and ability of animals to obtain normal posture (signaling lack of effect on the sensory statocyst apparatus) at very high exposure levels.

IS PRESSURE OF PRIMARY CONCERN FOR BIOLOGICAL INJURY?

Sound waves having high peak pressures have generally been the focus of major concern with respect to the potential effect of seismic surveys on aquatic organisms. However, the relative importance of peak pressure and total cumulative energy is unknown, especially in relation to subtle but potentially injurious sub-lethal effects which have not been investigated to any extent.

Some effects could be linked to peak pressure while others could be linked to total cumulative energy. For instance, mortality of an immediate or very short term nature as seen in a few studies to date could be more or less linked to peak pressure while more subtle effects of which we are unaware such as chronic over-stimulation of neuroendocrine systems or for instance mechano-sensory systems, such as sensitive cilia in ears and lateral lines (of fish but analogs also exist across a wide range of species), could also be occurring and linked to cumulative energy. Of course, it would be difficult to separate one from the other with both having a role even in the case of one particular type of injury. For instance, acute and immediate injury to mechano-sensory systems might be primarily due to high peak pressures while additional injury might stem from more chronic exposure to much lower peak pressures. Simply put, longer term exposures to low intensity sounds could be just as important as short term exposure to very loud sounds. It would all depend on the type of biological perturbation or (perhaps) injury under consideration. For instance drawing on examples from model mammalian systems, chronic low level noise has been shown to produce blood disorders (Bijlsma et al. 2001) and modulate cellular transport functions (Chohan et al. 1984), to name a few effects.

There is also the question of whether particle motion might have played a role in the physiological/cellular disturbances observed in lobster in the present study since particle motion would be expected to be greater in exposures at close range for a given pressure level. Many fish appear to be able to detect particle motion (e.g. Popper et al 2003) but it is not known if air gun induced particle motion alone can be considered to be an important risk factor for producing injuries or potentially injurious effects in the organs of fish and shellfish. Indeed it would seem surprising if this were found to be the case.

FUTURE DIRECTIONS

Exposure of lobster to peak-to-peak sound pressure levels around 202 dB has been shown to have no apparent effect on such important endpoints as delayed mortality, or damage to mechano-sensory systems associated with animal equilibrium and posture. However, sub-lethal effects have been observed with respect to feeding and serum biochemistry. An interesting effect was also observed in histological studies of the hepatopancreas of animals which had been exposed some months previously.

These initial studies were meant to be exploratory in nature and caution is warranted about over-interpretation. However, they do point to the need for more comprehensive studies regarding the potential for seismic surveys to affect lobster. A few experiments with lobster carried out along the lines of an actual seismic survey and employing different energy levels (vis a vis in relation to water depth) would be prohibitively expensive (certainly in the millions of dollars range). However, biological impact studies can reasonably be addressed in a

cost effective manner following the approach taken here, through use of large aquaria and small scale field exposures, with animals being subsequently retained in the laboratory for observation. The results of the present research also indicate that sub-lethal effects may be delayed and/or persist to varying degrees after animal exposures. This indicates the importance of periodic assessment of various parameters which would not be logistically feasible after a field survey type experiment where animals would have to be left on the sea-bottom for several months. Also, any repeated lifting of animals from the sea-bottom for sampling could introduce serious artifacts for many physiological parameters. Furthermore, important endpoints like feeding which can affect growth and reproduction could not be assessed at all. Starvation of animals through holding in cages would also greatly compromise histopathological criteria. Also, given the potential for variability of response throughout the year with respect to feeding, moulting etc., replicate field trials would be required. This is not to say that opportunistic "monitoring" studies carried out during an authentic field study would not be important, but it is difficult to determine at this time what should be monitored in the absence of more comprehensive experimental studies or indeed if monitoring studies are warranted at all.

We note the advantage of the approach taken in this pilot study with lobster in relation to cost, feasibility and scientific validity. It is recognized that when experiments are carried out in large tank systems, sound may be reflected to a degree whereby animals might receive somewhat "extra" energy. However, the major impulse (and its associated metrics) can reasonably be considered to be of primary importance when considering approximations for biological effects. It is also reasonable to note in this regard that many bottom living animals such as lobster reside in rocky habitats of complex geometry which can vary from place to place even over a range of a meter or so, and for which sound reflections would all be expected to vary to a degree.

RECOMMENDATIONS

This pilot study with lobster established feeding, hemolymph and histological effects in animals exposed to relatively low levels of sound with some effects being observed weeks to months after the exposures. The noted effects may be linked to disturbances (or injury?) to neural/endocrine systems or membrane transport functions. Accordingly, it is recommended that priority be given to key effects which might be linked to such disturbances yet regarded as injurious. These include (a) animal moulting with select studies on feeding and pathology (b) egg development and (c) lobster behavior and movement. Studies on moulting would include studies near the beginning of the moulting period to assess inhibitory or stimulating effects as well as investigation for effects on animals at the "soft" stage of moulting itself. Animals would be initially exposed in the laboratory to pressure levels around 200 dB and varying numbers of shots. Should effects be observed, energy levels would be lowered in stepwise fashion

to obtain an appreciation of "no-effects levels". Such experiments and especially with "soft" animals would be logistically difficult with respect to exposures, laboratory holding and overall animal husbandry but moulting has potential to be a very sensitive endpoint and warrants priority investigation.

Effects on egg development and animal behavior would be investigated in exposures carried out in the field. The exposures would be carried out with a towed sound source providing representative energy levels found as a continuum under seismic surveys. Received sound pressure levels would be measured at various locations with lobster movement studied by telemetry using coded acoustic tags. Exposures would be carried out during both day and night, since lobsters move from their shelters and are typically more active at night. Also received energy levels could be quite different between animals outside and inside shelters (pilot studies on which would also be investigated in the laboratory). Lobster position resolution would be about 2m within the area bounded by hydrophones and receivers would be placed on buoys to collect transmitter data from an animal being tracked. Pre-exposure tracking studies would be required to identify "normal" activity trends.

Animals would be placed in cages for the egg development studies and exposed in the same manner as for the telemetric studies e.g. moving sound source. Animals would be returned upon exposure to aquaria and held for several months with selected indices being assessed on a regular basis.

The described studies can be carried out in a cost effective manner through laboratory and small field experiments as used in the pilot study reported here. It is further concluded that such studies would be in agreement with the precautionary principle and go a long way in providing information of importance for assessing if seismic surveys pose a significant risk to lobster.

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Table 1. Sound metrics at the cage site in the aquarium.

Calibration 1	Average	Standard	Maximum	Minimum	Units
SPL Peak to Peak	202.312	0.586	203.374	199.553	dB re 1uPa
SPL Peak	197.557	0.744	198.713	193.931	dB re 1uPa
SPL RMS	176.322	0.679	178.125	174.272	dB re 1uPa
EDS Max Peak	144.952	1.855	149.372	137.762	dB re 1 uPa^2/Hz
EDS Peak Frequency	24.172	0.58	25.5	22.5	Hz
Sound Exposure Level	178.87	1.359	182.477	174.771	dB re 1uPa^2 * sec

Calibration 2	Average	Standard	Maximum	Minimum	Units
SPL Peak to Peak	203.472	0.504	204.231	202.214	dB re 1uPa
SPL Peak	198.294	0.835	199.296	196.236	dB re 1uPa
SPL RMS	182.946	1.574	185.39	180.351	dB re 1uPa
EDS Max Peak	169.479	3.942	175.828	161.895	dB re 1 uPa^2/Hz
EDS Peak Frequency	25.351	0.652	27.5	24	Hz
Sound Exposure Level	192.118	3.147	197.006	186.929	dB re 1uPa^2 * sec

Calibration 3	Average	Standard	Maximum	Minimum	Units
SPL Peak to Peak	202.326	0.67	203.597	198.994	dB re 1uPa
SPL Peak	196.953	0.783	198.713	193.821	dB re 1uPa
SPL RMS	176.573	0.592	178.295	174.545	dB re 1uPa
EDS Max Peak	147.291	1.729	153.37	143.383	dB re 1 uPa^2/Hz
EDS Peak Frequency	21.182	2.71	31	18.5	Hz
Sound Exposure Level	179.373	1.184	182.816	175.317	dB re 1uPa^2 * sec

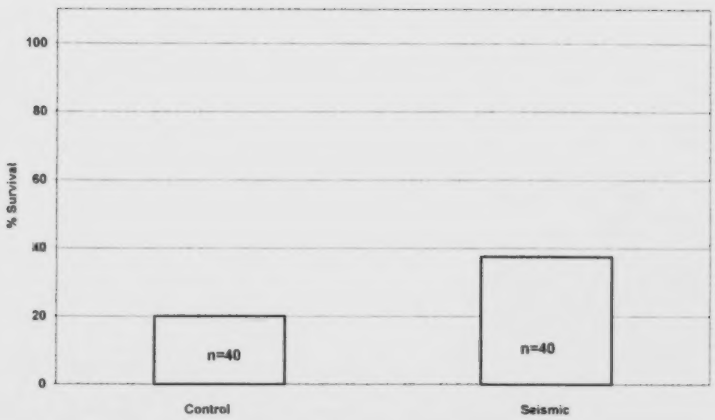


Figure 1. Percent survival 8 months post exposure Trial 1 (Exposure 0.5°C, ~202 dB p-p, 30 shots).

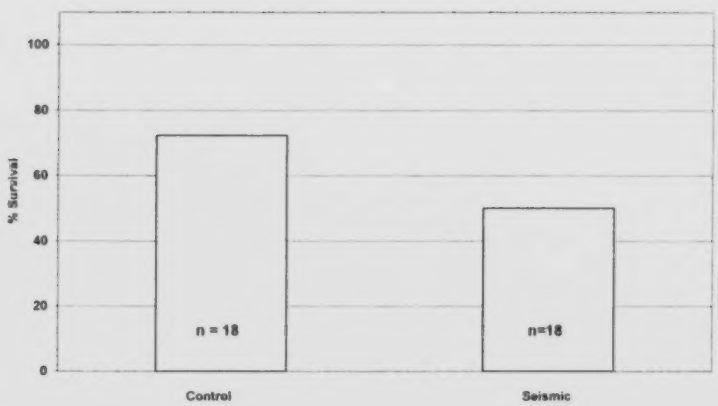


Figure 2. Percent survival 4 months post exposure Trial 2 (Exposure 0.5°C, ~202 dB p-p, 30 shots).

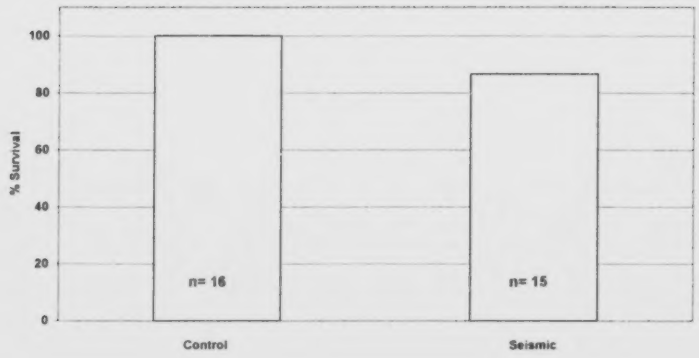


Figure 3. Percent survival 4 months post exposure Trial 3 (Exposure 14.0°C, ~202 dB p-p, 20 shots).

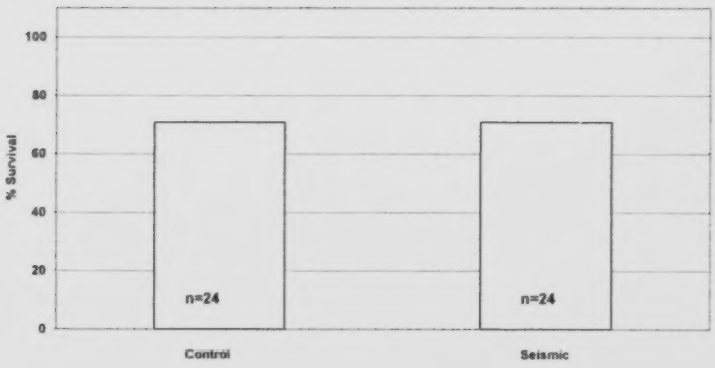


Figure 4. Percent survival 9 months post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

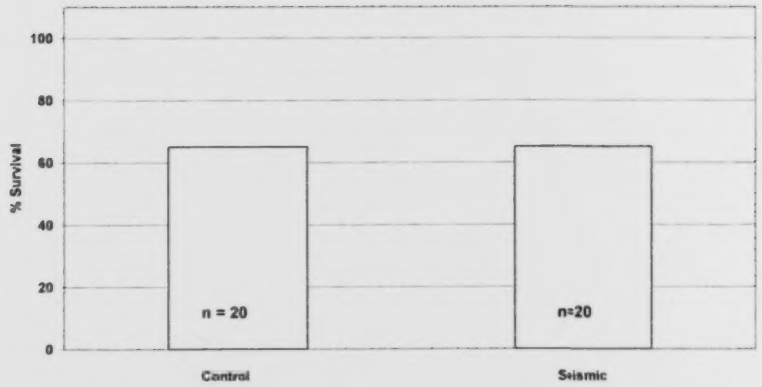


Figure 5. Percent survival 6 months post exposure Trial 5 (Exposure 1.0°C, ~202 dB p-p, 200 shots).

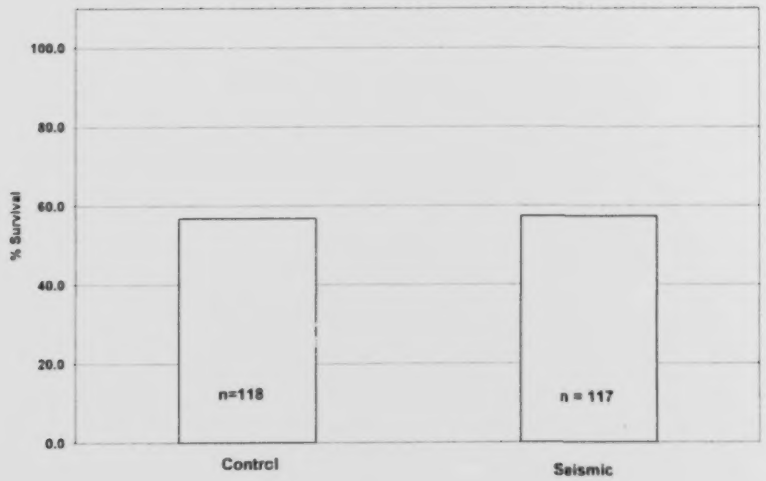


Figure 6. Percent survival; Total for all 5 trials.

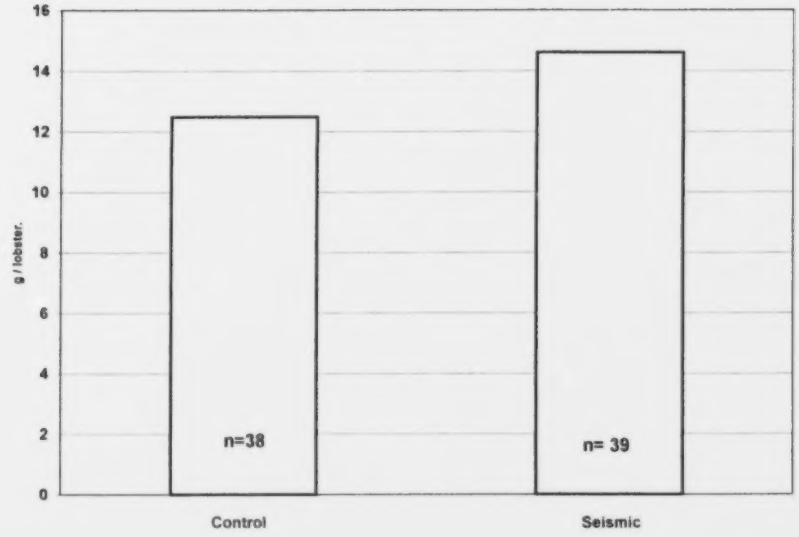


Figure 7. Food consumption (g/lobster) 15 days post exposure Trial 1 (Exposure 0.5°C, ~202 dB p-p, 30 shots).

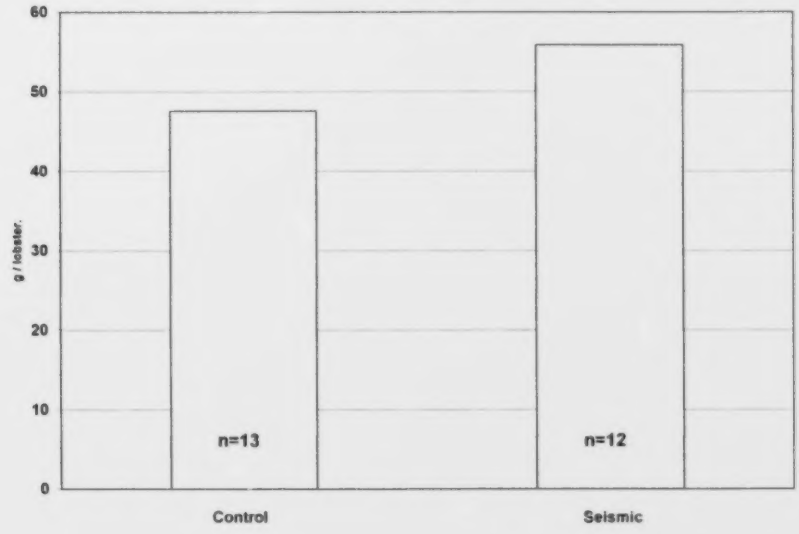


Figure 8a. Food consumption (g/lobster) 69 days post exposure Trial 2 (Exposure 0.5°C, ~202 dB p-p, 30 shots).

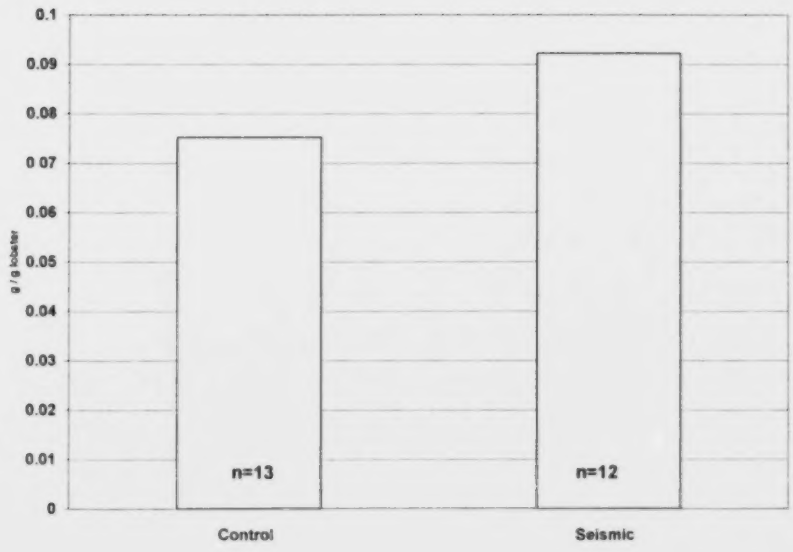


Figure 8b. Food consumption (g/g lobster) 69 days post exposure Trial 2 (Exposure 0.5°C, ~202 dB p-p, 30 shots).

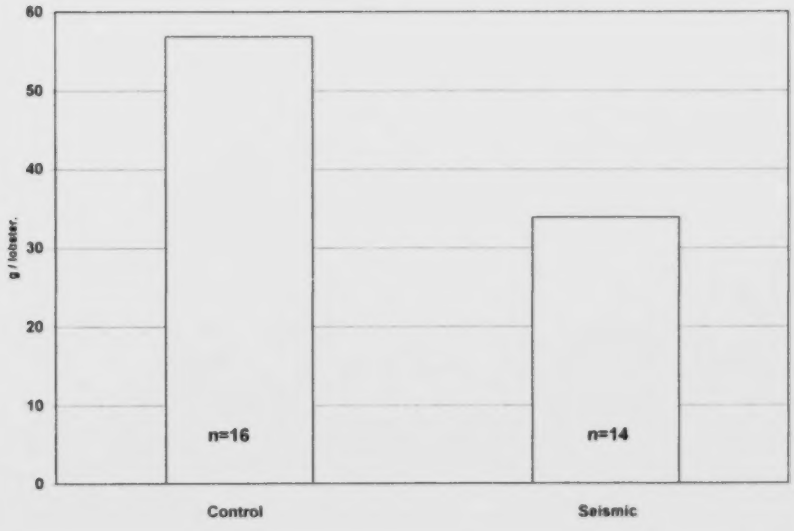


Figure 9a. Food consumption (g/lobster) 8 days post exposure Trial 3 (Exposure 14.0°C, ~202 dB p-p, 20 shots).

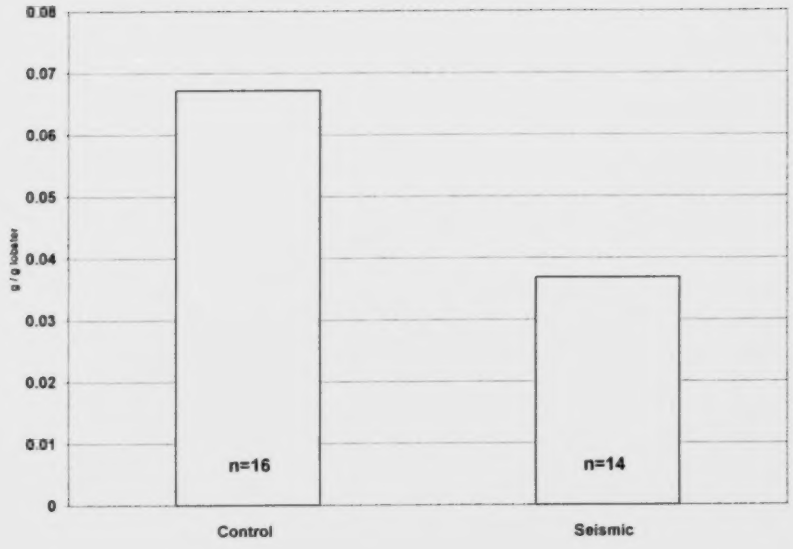


Figure 9b. Food consumption (g/g lobster) 8 days post exposure Trial 3 (Exposure 14.0°C, ~202 dB p-p, 20 shots).

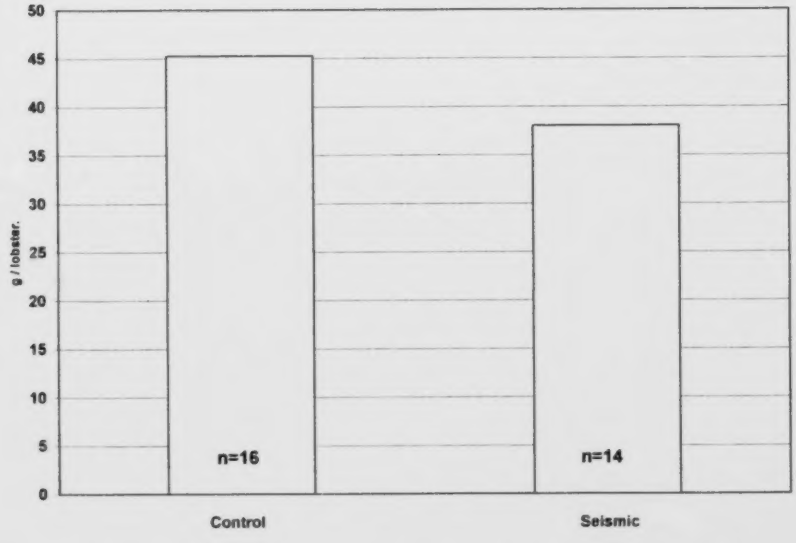


Figure 10a. Food consumption (g/lobster) 43 days post exposure Trial 3 (Exposure 14.0°C, ~202 dB p-p, 20 shots).

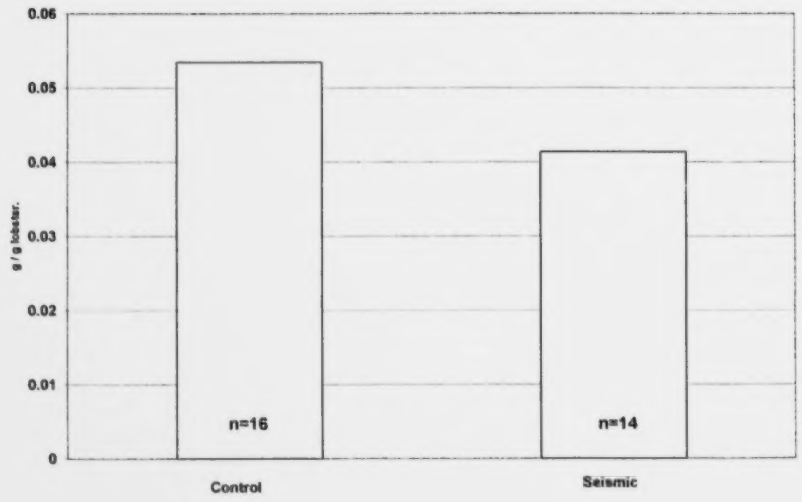


Figure 10b. Food consumption (g/g lobster) 43 days post exposure Trial 3 (Exposure 14.0°C, ~202 dB p-p, 20 shots).

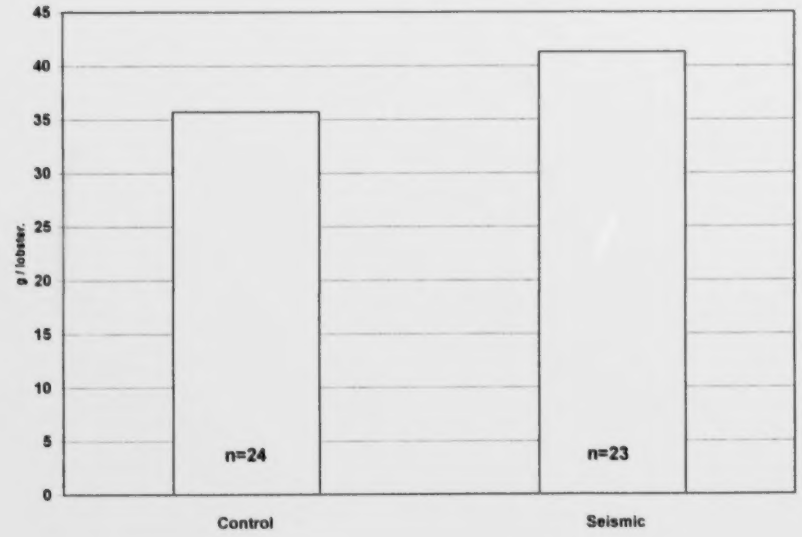


Figure 11a. Food consumption (g/lobster) 12 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

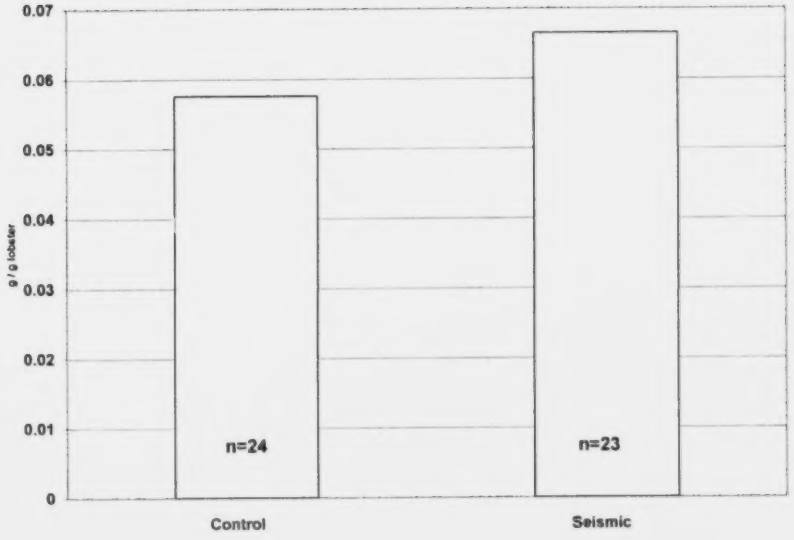


Figure 11b. Food consumption (g/g lobster) 12 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

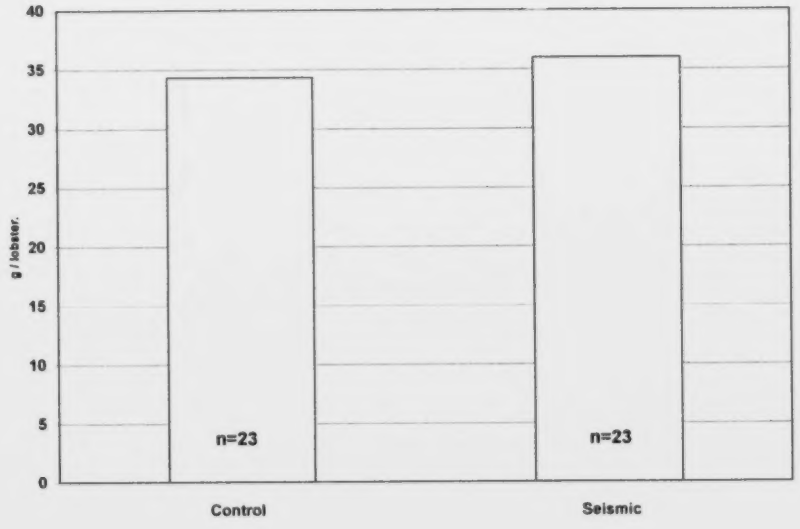


Figure 12a .Food consumption (g/lobster) 40 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

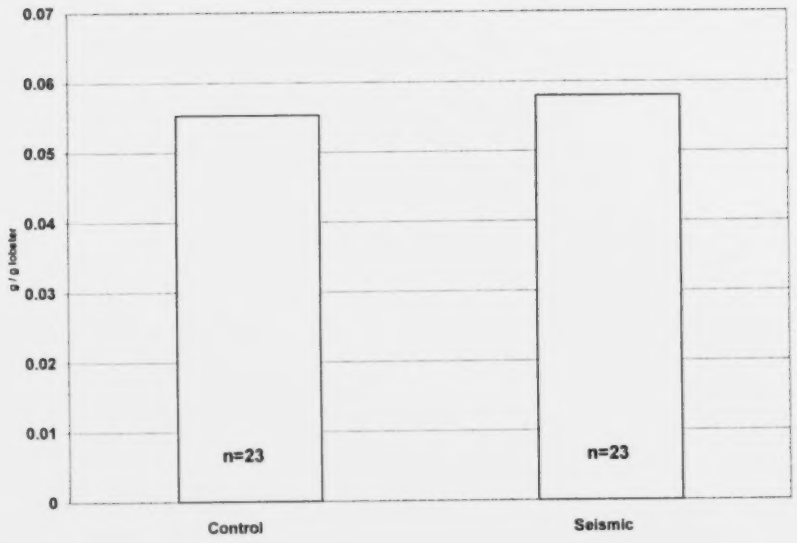


Figure 12b. Food consumption (g/g lobster) 40 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

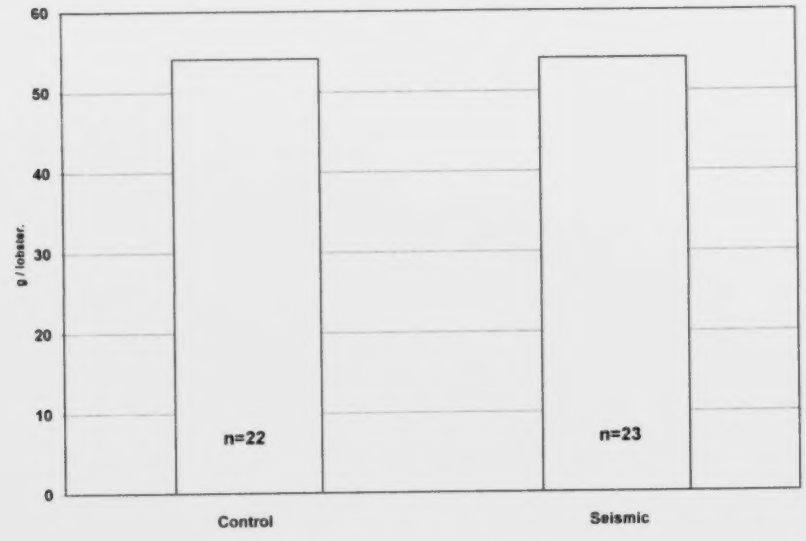


Figure 13a. Food consumption (g/lobster) 162 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

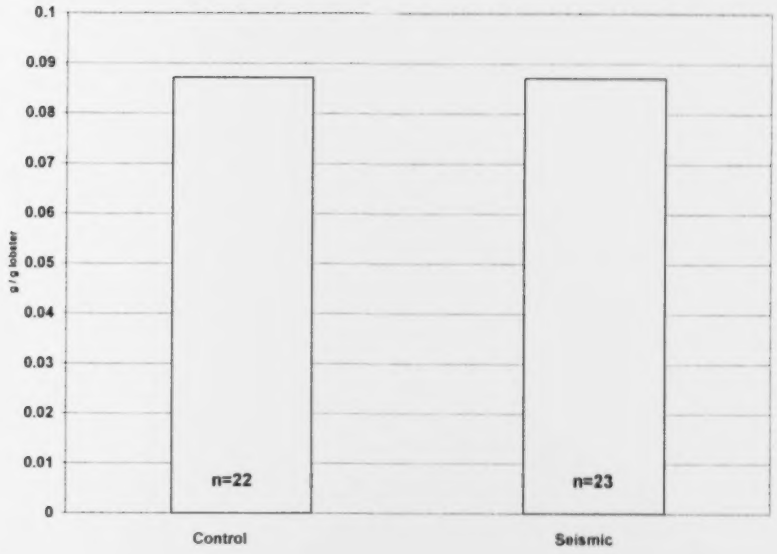


Figure 13b. Food consumption (g/g lobster) 162 days post exposure Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots).

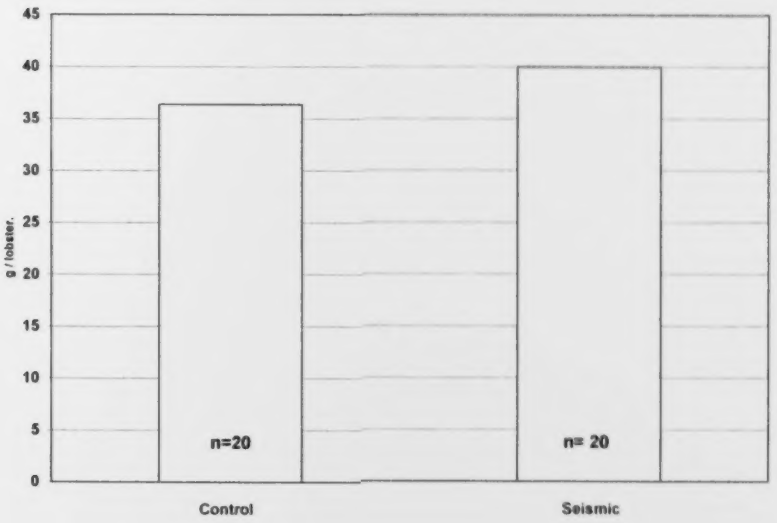


Figure 14a. Food consumption (g/lobster) 20 days post exposure Trial 5 (Exposure 1.0°C, ~202 dB p-p, 200 shots).

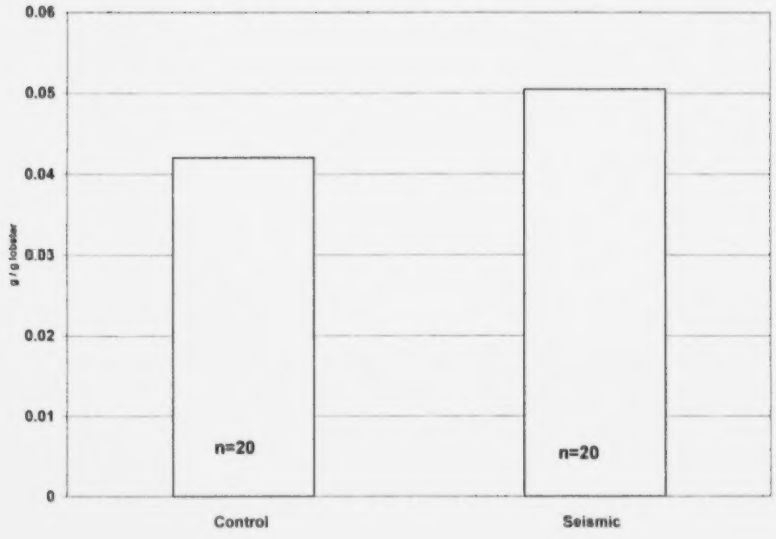


Figure 14b. Food consumption (g/g lobster) 20 days post exposure Trial 5 (Exposure 1.0°C, ~202 dB p-p, 200 shots).

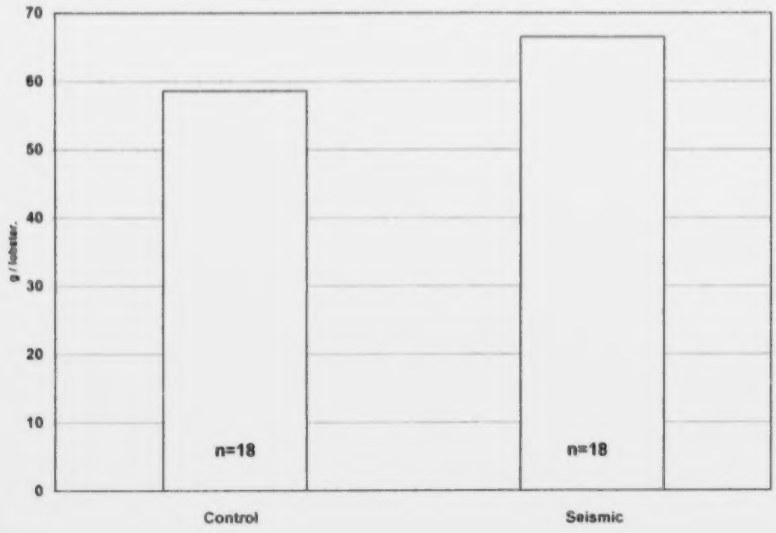


Figure 15a. Food consumption (g/lobster) 77 days post exposure Trial 5 (Exposure 1.0°C, ~202 dB p-p, 200 shots).

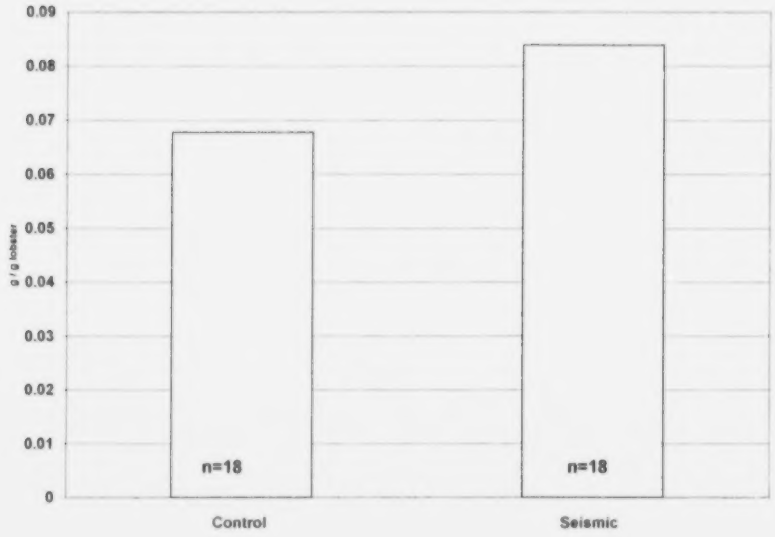


Figure 15b. Food consumption (g/g lobster) 77 days post exposure Trial 5 (Exposure 1.0°C, ~202 dB p-p, 200 shots).

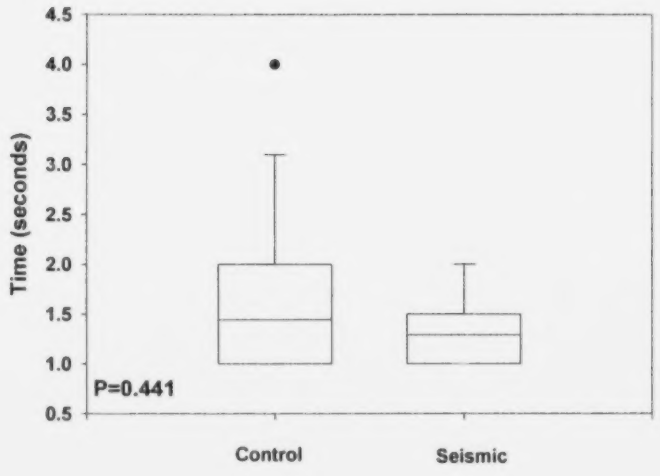


Figure 16. Differences in turnover rate in Trial 1 (Exposure 0.5°C, ~202 dB p-p, 30 shots) on Day 142 after second seismic exposure.

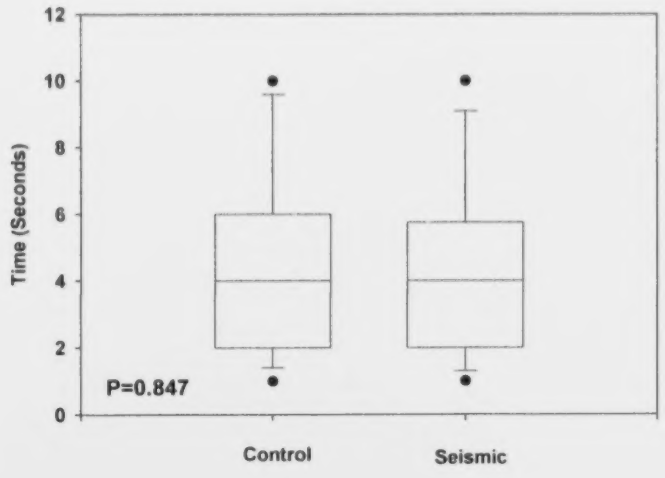


Figure 17. Differences in turnover rate in Trial 2 (Exposure 0.5°C, ~202 dB p-p, 30 shots) on Day 65.

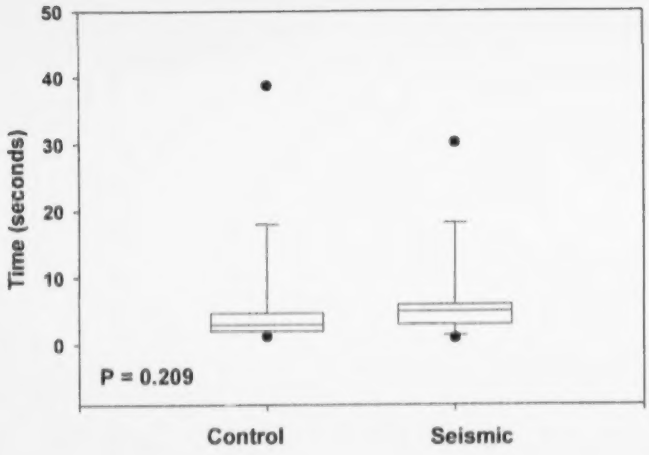
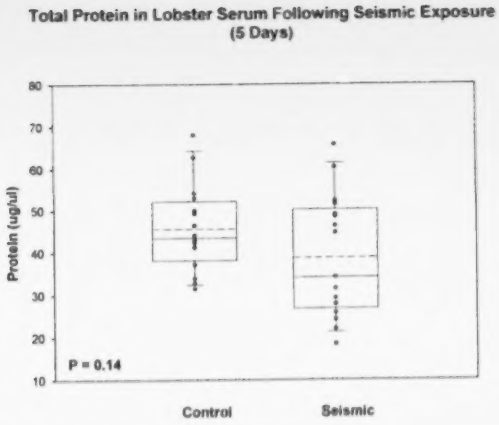
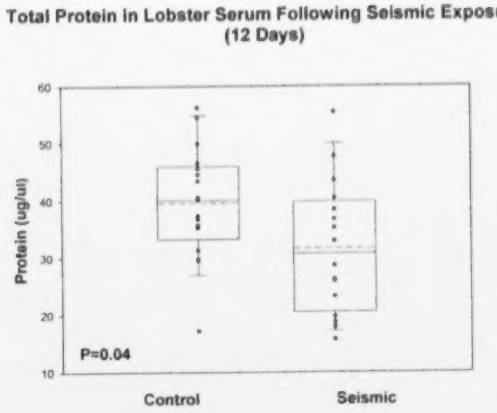


Figure 18. Differences in turnover rate in Trial 4 (Exposure 6.0°C, ~227 dB p-p, 50 shots) on Day 9.

a.



b.



c.

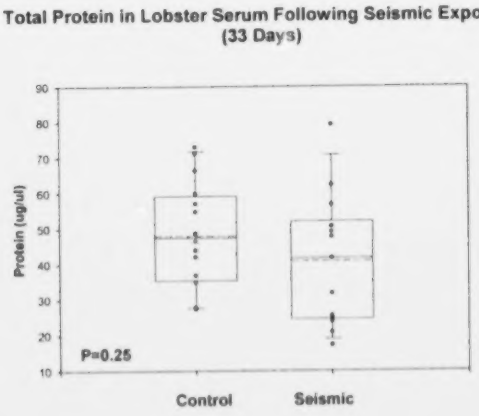


Figure 19. Effect on serum protein in lobster from **Trial 2**, (Exposure 0.5°C; ~202 dB p-p, 30 shots); a, b and c indicate days post exposure.

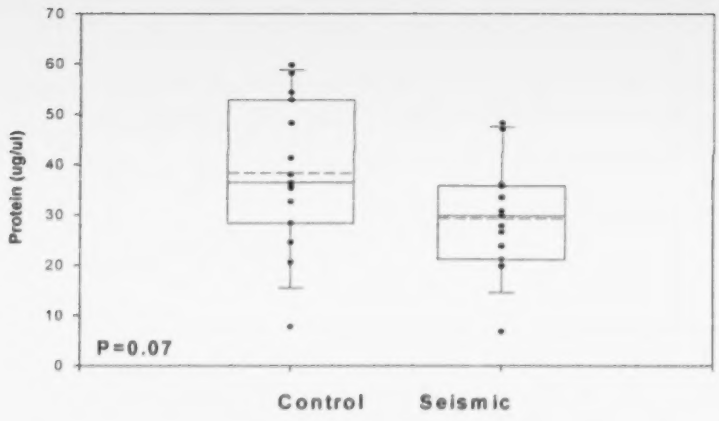


Figure 20. Effect on serum protein on Day 2 in lobster from **Trial 3**, (Exposure 14.0°C; ~202 dB p-p, 20 shots).

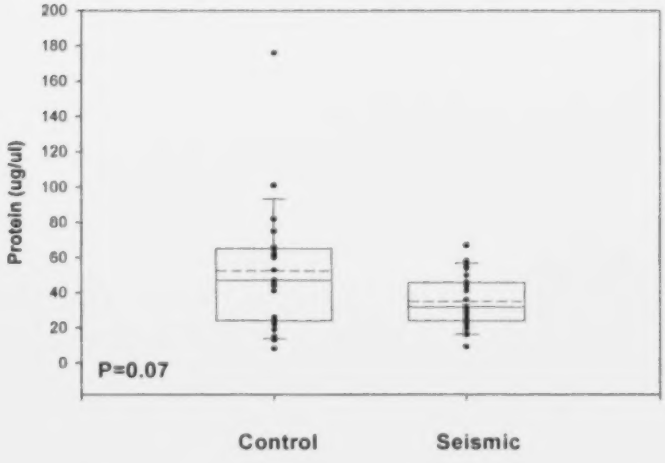


Figure 21. Effect on serum protein on Day 5 in lobster from **Trial 4**, (Exposure 6.0°C; ~227 dB p-p, 50 shots).

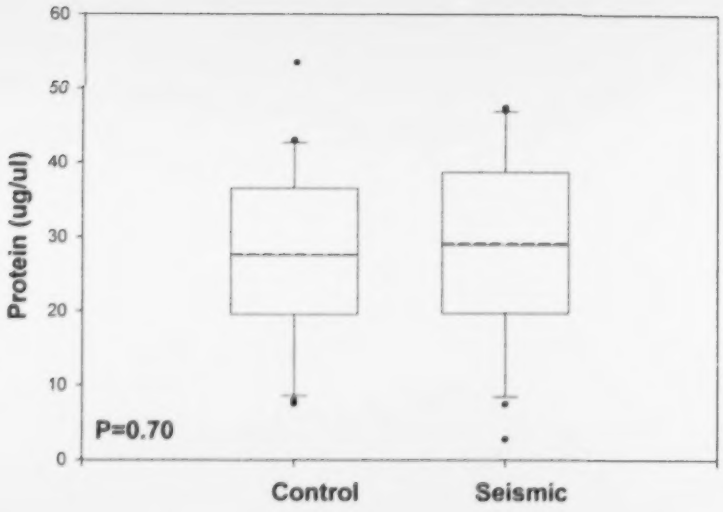
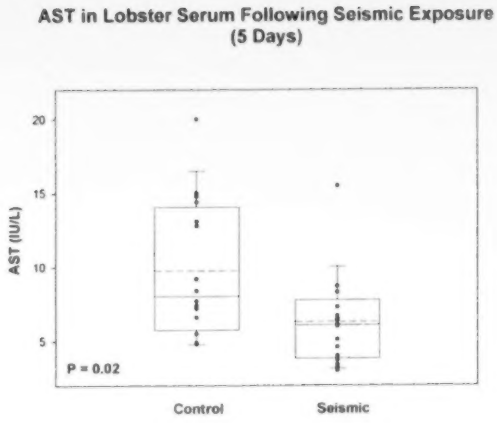
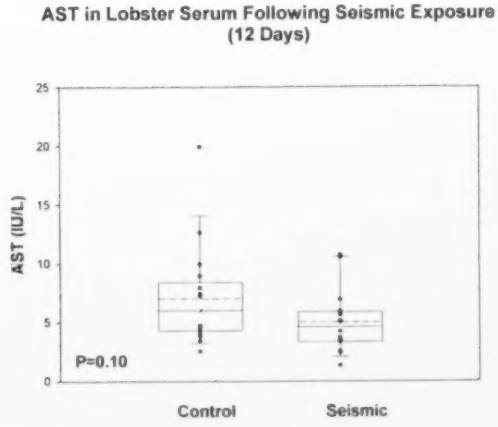


Figure 22. Effect on serum protein on Day 5 in lobster from **Trial 5,** (Exposure 0.5°C; ~202 dB p-p, 200 shots).

a.



b.



c.

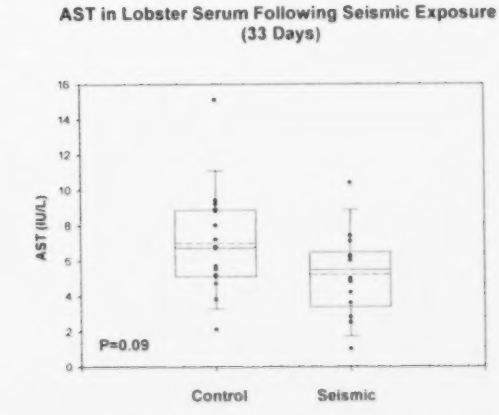


Figure 23. Effect on serum AST in lobster from **Trial 2,** (Exposure 0.5°C; ~202 dB p-p, 30 shots); a, b and c indicate days post exposure.

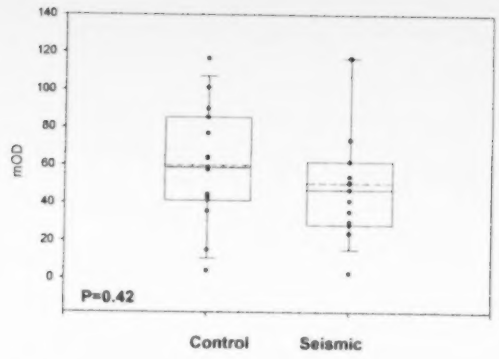


Figure 24. Effect on serum AST on Day 2 in lobster from **Trial 3,** (Exposure 14.0°C; 202 dB p-p, 20 shots).

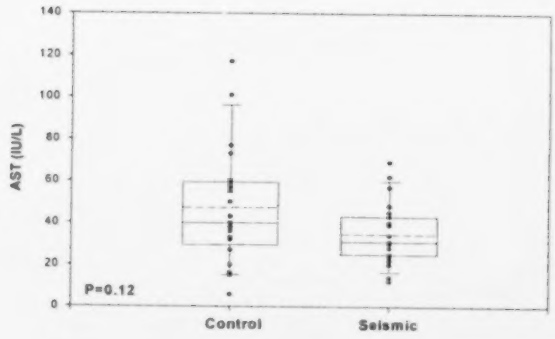


Figure 25. Effect on serum AST on Day 5 in lobster from **Trial 4,** (Exposure 6.0°C; ~227 dB p-p, 50 shots).

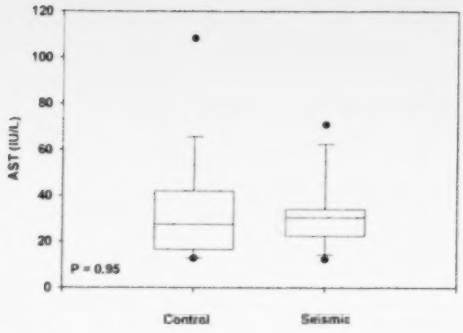
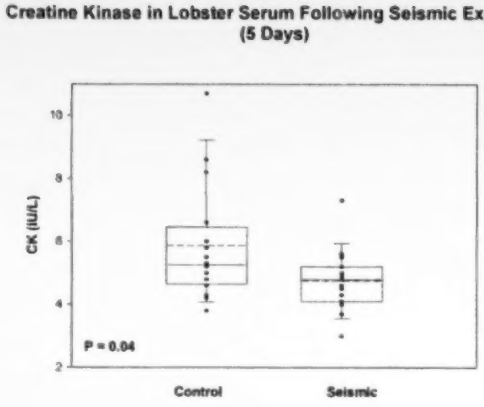
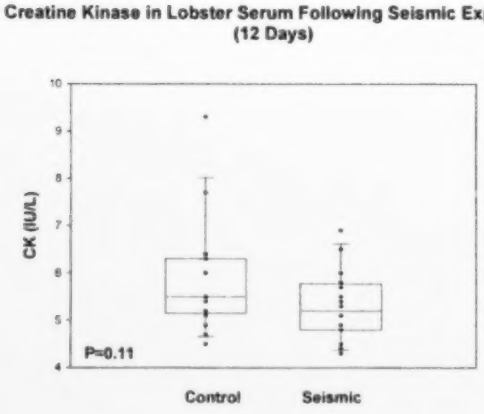


Figure 26. Effect on serum AST on Day 5 in lobster from **Trial 5,** (Exposure 0.5°C; ~202 dB p-p, 200 shots).

a.



b.



c.

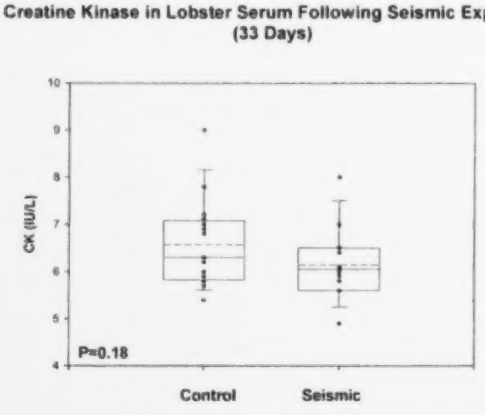


Figure 27. Effect on serum CK in lobster from **Trial 2,** (Exposure 0.5°C; ~202 dB p-p, 30 shots); a, b and c indicate days post exposure.

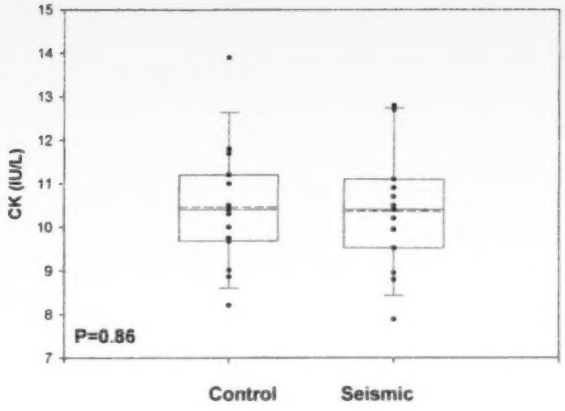


Figure 28. Effect on serum CK on Day 2 in lobster from **Trial 3**, (Exposure 14.0°C; ~202 dB p-p, 20 shots).

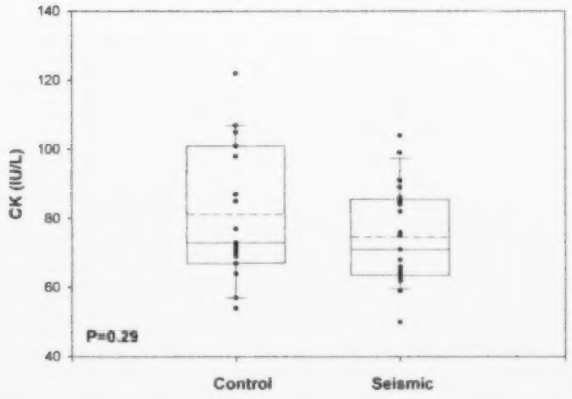


Figure 29. Effect on serum CK on Day 5 in lobster from **Trial 4**, (Exposure 6.0°C; ~227 dB p-p, 50 shots).

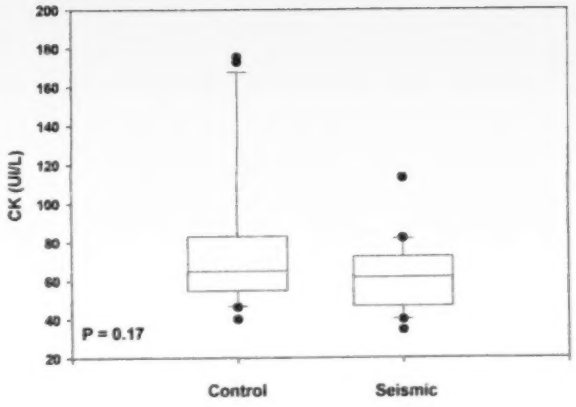
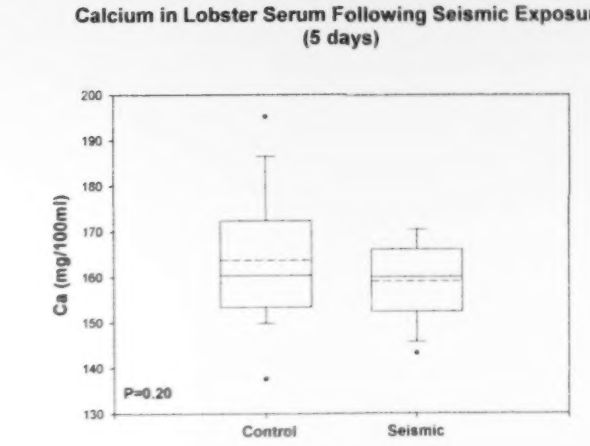


Figure 30. Effect on serum CK on Day 5 in lobster from **Trial 5**, (Exposure 0.5°C; ~202 dB p-p, 200 shots).

a.



b.

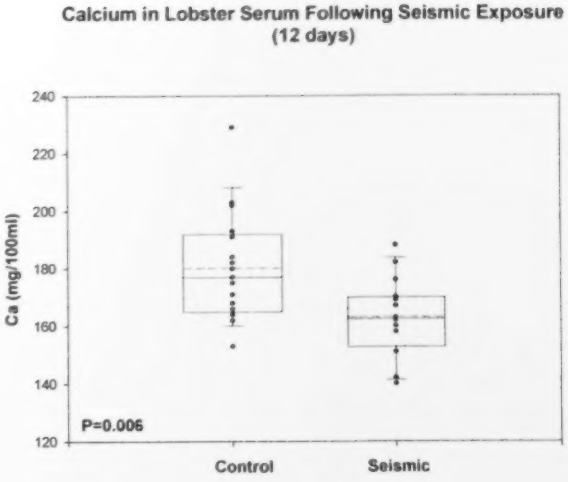


Figure 31. Effect on serum calcium in lobster from **Trial 2**, (Exposure 0.5°C; ~202 dB p-p, 30 shots); a and b indicate 5 and 12 days post exposure respectively.

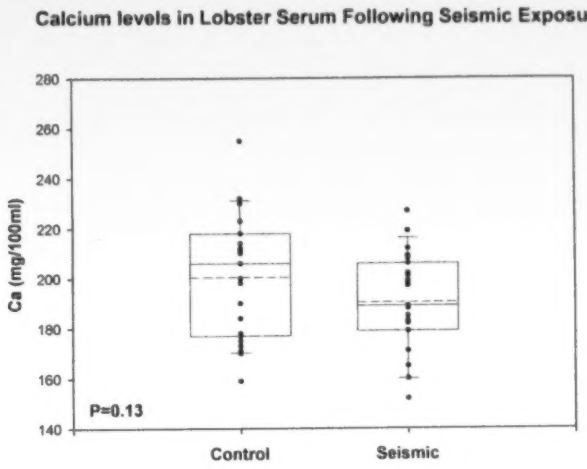
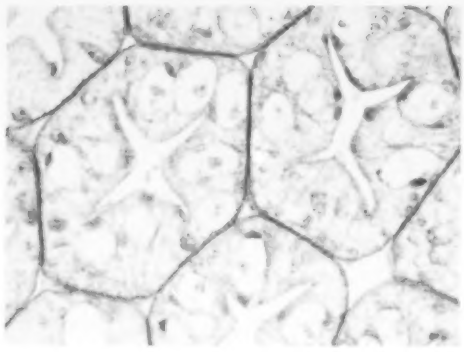


Figure 32. Effect on serum calcium on Day 5 in lobster from **Trial 4**, (Exposure 6.0°C; ~227 dB p-p, 50 shots).

a.

CONTROL



b.

SEISMIC EXPOSED

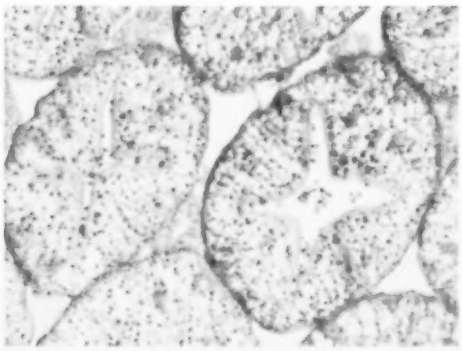


Figure 33. Histochemical differences in the hepatopancreas 4 months post exposure **Trial 2**, (Exposure 0.5°C; ~202 dB p-p, 30 shots); a. Control, b. Exposed. The dots in the exposed group represent deposits of PAS staining material. The control group contained essentially no PAS staining material, as noted here, or much lower levels.

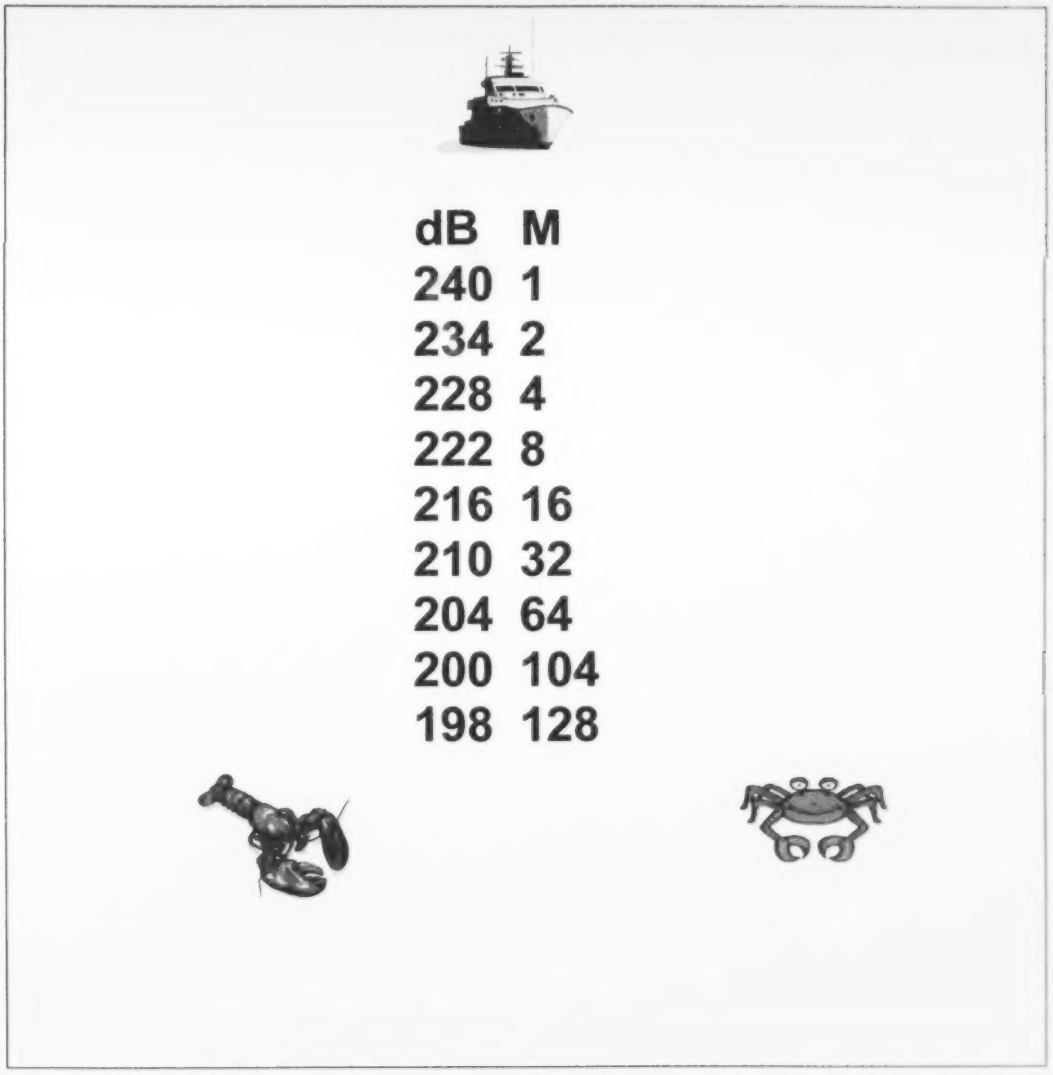


Figure 34. Hypothetical distances for sound travel down into the water column assuming spherical spreading.